Guidance for Industry

Antiviral Drug Development — Conducting Virology Studies and Submitting the Data to the Agency

DRAFT GUIDANCE

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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

May 2005
Clinical Antimicrobial

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I. INTRODUCTION

The purpose of this guidance is to assist sponsors in the development of antiviral drug products and to serve as a starting point for understanding the nonclinical and clinical virology data important to support clinical trials of antiviral agents. This guidance focuses on nonclinical and clinical virology reports, which are essential components in the review of investigational antiviral drugs. Topics in this guidance include studies defining the mechanism of action, establishing specific antiviral activity of the investigative drug, providing data on the development of viral resistance to the investigational drug, and providing data identifying cross-resistance to approved drugs having the same target.

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II. BACKGROUND

The recommendations in this guidance are based on the review experience with antiviral drugs of the Division of Antiviral Drug Products (DAVDP) as well as input from pharmaceutical sponsors and the scientific community. Because of the experience, history, and lessons learned with HIV-1 studies, this guidance focuses on studies commonly used to evaluate HIV-1 drugs and uses them as a paradigm for future studies of drugs to treat other viruses. Since the field of

[1] This guidance has been prepared by the Division of Antiviral Drug Products in the Center for Drug Evaluation and Research (CDER) at the Food and Drug Administration.

virology is dynamic and continually evolving, this guidance will be revised as new information accumulates and as circumstances warrant.

Guidances on the overall organization of investigational new drug (IND) applications and new drug applications (NDAs) can be found at www.fda.gov/cder/regulatory /applications/default.htm. Sponsors are encouraged to contact the Agency early in the development of an investigational antiviral drug to facilitate the review and approval process. To assist prospective sponsors, the Agency accepts informal submissions in an abbreviated IND format (i.e., pre-INDs) for review and comment. Pre-INDs are especially useful in instances where applicants are unfamiliar with the process for evaluating investigational drug products in humans. Information about submitting pre-INDs can be found at www.fda.gov/cder/ode4/preind/getting.htm. The Agency accepts electronic submissions to expedite the review process. FDA Web sites can be consulted for information on electronic application submission.

III. NONCLINICAL VIROLOGY REPORTS

A. Overview

Nonclinical virology reports aid in evaluating the safety and efficacy of an investigational drug before it is tested in humans. The reports can include studies identifying the mechanism of action, establishing specific antiviral activity of the compound in a model system, and providing data on the development of viral resistance to the investigational drug. Additionally, co-administration of an investigational drug with other drugs approved for the same indication is often necessary to treat virus infections in clinical settings. In these cases, it is desirable to have in vitro combination activity studies designed to identify possible negative interactions on antiviral activity (i.e., antagonism) of the investigational drug with other antiviral drugs.

As more antiviral drugs are developed to treat particular viral diseases, cross-resistance (i.e., viral resistance to one drug causing resistance to more than one agent within that drug class) can become a major issue in clinical settings. Therefore, from a scientific perspective, the following information is critical in the development of antiviral drug products:

- Determining the antiviral activity of an investigational drug against viruses resistant to other drugs with the same molecular target

- Determining the antiviral activity of approved drugs against viruses resistant to the investigational drug with the same molecular target

Specific details of each of the nonclinical studies recommended by the FDA can be found in Section III.B., Recommended Components of Nonclinical Virology Reports. We recommend conducting nonclinical studies (i.e., mechanism of action, antiviral activity in vitro, cytotoxicity/therapeutic index, and effects of serum protein binding on antiviral activity) before the initiation of phase 1 clinical studies. We suggest that sponsors complete in vitro drug combination activity studies of the investigational drug with the approved drugs before the

initiation of clinical trials that will examine the efficacy of the investigational drug in combination with approved drugs. Furthermore, we recommend examining the in vitro selection of resistant viruses to the investigational drug, the phenotypic and genotypic characterization of the resistant viruses, and cross-resistance analyses before initiation of clinical studies in patients infected with the particular virus. Complete virology study reports on nonclinical and clinical studies can be submitted upon their completion and need not be held until submission of the NDA.

B. Recommended Components of Nonclinical Virology Reports

1. Mechanism of Action

We prefer that mechanism of action studies be conducted before the initiation of phase 1 clinical studies. Nonclinical virology reports on the mechanism of action should include background information and data identifying the mechanism of action of the investigational drug and its metabolites. There are many steps in a virus life cycle that can be targeted by potential drug candidates. Compounds can act directly to inhibit a virus by targeting a specific viral-encoded function (e.g., an enzyme inhibitor) or act indirectly (e.g., interferon induction of host cell response). We recommend that mechanism of action studies:

- Demonstrate the investigational drug's ability to specifically inhibit viral replication or a virus-specific function

- Establish the site of the drug's action (e.g., viral replicase, integrase, protease)

Biochemical, structural, cellular, or genetic data can be presented to support the proposed mechanism of action. Data that demonstrate the mechanism of action include, but are not limited to, receptor binding, inhibition of enzymatic activity, X-ray crystallographic structure determination of bound inhibitor complex, and characterization of resistance mutations in the gene encoding the target.

A well-characterized mechanism of action is useful in predicting toxicities and in designing studies to assess the development of resistance. A clear understanding of the mechanism of action for an investigational antiviral drug can provide insight into the regions of the viral genome where resistance mutations could develop. These regions are not limited to the site of action (viral-encoded target) of the investigational drug but can include the enzyme substrate or another viral-encoded protein existing in a complex with the target protein. Characterization of the resistance mutations can provide in vivo validation of the mechanism of action studies.

The specificity of the investigational drug should be demonstrated for the viral target over cellular/host proteins, especially in those cases in which a viral enzyme has a cellular counterpart. For example, if the investigational drug targets a viral polymerase, we recommend showing the activity of the drug against the viral polymerase in comparison with its activity against host DNA polymerases such as DNA Polymerase α, β, and γ.

Immunomodulatory drugs raise additional issues. These drugs can have the potential for unintended adverse effects resulting from the drug's actions on the immune system in addition to effects on viral replication. We recommend that an indirect antiviral effect and the specific immune system components that are involved in the drug's action be identified for investigational drugs that act through a general immune stimulatory mechanism. We recommend consulting the DAVDP for specific advice regarding the development of immunomodulatory drugs for the treatment of viral diseases.

2. Antiviral Activity

a. Antiviral activity in vitro

For many human viruses, there are cell culture systems or animal hosts in which the infectious agent can undergo a complete virus life cycle. In these cases, we recommend that the sponsor document that the investigational drug and/or its metabolites show specific, quantifiable antiviral activity in vitro before initiating tests in humans (i.e., before initiation of phase 1 studies). It is important that these data support clinical testing in humans by providing clear evidence of antiviral effects at drug concentrations that can be achieved in vivo with acceptable risk-benefit. Additionally, in vitro antiviral activity and cytotoxicity assessments (see Section III.B.3., Cytotoxicity/Therapeutic Index) can be used to guide the selection of appropriate dose ranges in early clinical trials by establishing a dose/response relationship using a broad range of relevant cell types and virus isolates. If possible, sponsors are encouraged to obtain antiviral activity data using primary human target cells. Because of viral genetic variation, the antiviral activity of the investigational drug should be examined for multiple clinical isolates and viral isolates representative of the virus population in clinical trials. Antiviral activity evaluations that are recommended to support the development of the investigational drug include:

- Assessing specific antiviral activity of the investigational drug against a broad range of clinical and laboratory viral isolates including different clades, subtypes, or genotypes

- Evaluating the antiviral activity of the investigational drug against mutant viruses that are resistant to drugs with the same molecular target as the investigational drug as well as viruses resistant to other drugs for the same indication

We recommend determining specific antiviral activity using a quantitative assay to measure virus replication in the presence of increasing concentrations of the drug compared to replication in the absence of drug. The inhibitory concentration (IC_{50}) is the concentration of the drug at which virus replication is inhibited by 50 percent. Assays that evaluate antiviral activity and cytotoxicity include but are not limited to virus inactivation assays, plaque reduction assays, cytopathic effect inhibition assays, peripheral blood mononuclear cell (PBMC) assays, and binding and fusion assays. Other factors that can be assessed in these assays include the effect of an increasing multiplicity of infection and the effect of pretreatment of virus or cells before infection versus treatment post-infection.

It is important that the inhibitory concentration be consistent with data supporting the mechanism of action. An investigational drug that inhibits virus replication at concentrations

lower than biochemical data for the proposed mechanism indicates that another target or mechanism of inhibition may be affected. For nucleoside or nucleotide analogs, we recommend that sponsors determine the intracellular half-life ($t_{1/2}$) of the triphosphate form of the active drug moiety in stationary and dividing cells from the target tissue.

For some human viruses (e.g., Hepatitis B and Hepatitis C viruses), no satisfactory cell culture or animal model exists, and in these cases, inhibition of an essential viral function or activity against related viruses can be used to indicate potential activity. When no satisfactory cell culture or animal model exists for the target human virus, it is particularly important to know whether or not a drug's active moiety enters cells, if it has a proposed intracellular site of action, and if the intracellular concentration is consistent with biochemical studies identifying an inhibitory concentration. Cell-based assays and host cell lines for studying viruses such as Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) replication may advance and improve, but at the present time are limited. For analysis of HCV replication, a replicon system has been developed that permits studies of viral replication and can be used to assess antiviral activity of some anti-HCV drugs. Currently, assays that examine HBV replication include, but are not limited to:

- Measuring HBV DNA polymerase activity in biochemical assays

- Cell culture assays with baculovirus-mediated cell transfer or transfection of HBV genomes into human hepatoma cell lines followed by quantitative Southern blot analysis with HBV DNA probes

- Cell culture assays with stably-transfected cell lines containing the HBV genome

- Quantitative PCR of extracellular HBV DNA

b. Antiviral activity in vitro in the presence of serum proteins

Serum proteins can bind to and sequester many drugs and may interfere with a drug's antiviral activity. We recommend that sponsors ascertain if the investigational drug is significantly bound by serum proteins. Common methods for determining protein binding include equilibrium dialysis, ultrafiltration methods, and fluorescence-based high throughput human serum albumin and α-acidic glycoprotein protein binding. If the investigational drug is highly protein bound, sponsors are encouraged to examine the in vitro antiviral activity of the investigational drug in the presence of increasing concentrations of human serum up to 40 to 50 percent. An IC_{50} for 100-percent human serum can be extrapolated from this data and the serum adjusted IC_{50} values reported. Sponsors are also encouraged to determine IC_{50} values in the presence of 2 mg/mL α-acidic glycoprotein.

c. Inhibitory quotient

Information on plasma and intracellular drug concentrations is important in assessing the dose/response of antiviral therapy and evaluating the potential for resistance development;

therefore, it is useful to determine an inhibitory quotient (IQ).[2] (For more information on determining IC_{50} values, see Section III.B.2.a., Antiviral activity in vitro.) We view IQ ratios as a useful tool integrating in vivo drug concentrations and antiviral activity. It is a measure that characterizes the relationship between drug exposure and the susceptibility of a virus to a drug. A high IQ indicates an effective drug concentration can be achieved in a patient to inhibit the virus and minimize the development of drug resistance. Since one dose may not be adequate for all patient populations, IQ ratios can be used to aid in the selection of a dose or doses to further evaluate in phase 3 clinical studies.

d. Antiviral activity in vivo

In cases where no cell culture or replicon system has been shown to be predictive of antiviral activity in humans, measurement of viral titers after drug treatment in animal model systems can be used to assess antiviral activity of the investigational drug. Analyses from animal models include the morbidity and mortality of animals following documented infection, histologic examination of tissues, quantification of viral titers over time, isolation and characterization of resistant isolates in animals that experience viral rebound, quantification of viral antigens and antibodies, pharmacokinetics of the investigational drug, and a description of symptoms (e.g., neurologic, weight loss). Antiviral drugs for smallpox and other orthopoxviruses require special considerations. Consult the DAVDP for guidance on the development of investigational drugs against orthopoxviruses.

3. Cytotoxicity/Therapeutic Index

It is important to establish that an investigational drug has antiviral activity at concentrations that can be achieved in vivo without inducing toxic effects to cells. Furthermore, in a cell culture model, the apparent antiviral activity of an investigational drug can result from host cell death after drug exposure. Cytotoxicity tests use a series of increasing concentrations of the antiviral drug to determine what concentration results in the death of 50 percent of the host cells. (See also Section III.B.2.a., Antiviral activity in vitro.) This value is referred to as the median cellular cytotoxicity concentration and is identified by CC_{50} or $CCIC_{50}$. The relative effectiveness of the investigational drug in inhibiting viral replication compared to inducing cell death is defined as the therapeutic or selectivity index (i.e., CC_{50} value/IC_{50} value). It is desirable to have a high therapeutic index giving maximum antiviral activity with minimal cell toxicity. We recommend determining CC_{50} values in both stationary and dividing cells from multiple human cell types and tissues for potential cell-cycle, species, or tissue-specific toxicities. We prefer that studies determining cytotoxicity/therapeutic index be conducted before the initiation of phase 1 clinical studies.

Because of the myelosuppressive effects of certain antiviral drugs, we recommend assessing the potential effects of certain candidate drugs on the growth of human bone marrow progenitor cells in colony formation assays. Some investigational antiviral drugs are potential inhibitors of DNA polymerases. The cellular DNA polymerases are responsible for normal nuclear and mitochondrial DNA synthesis and repair. The inhibition of human pol γ, the enzyme responsible

[2] The C_{min}/serum adjusted IC_{50} value.

for mitochondrial DNA synthesis, has been linked to defects in mitochondrial function. Therefore, it is important to monitor the effects of certain investigational drugs (e.g., nucleoside analogs) on mitochondrial toxicity by examining mitochondrial morphology, glucose utilization, lactic acid production, and mitochondrial DNA content.

4. In Vitro Combination Activity Analysis

Within an infected individual, viruses can exist as a heterogeneous population of variant viruses, some of which may show reduced susceptibility to one or more antiviral drugs. Therefore, for some viruses, administration of multiple antiviral drugs (e.g., three-drug combination antiretroviral therapy against HIV-1) can be more effective than a single drug in establishing and maintaining inhibition of virus replication. However, the interactions of drugs are complex and can result in antagonistic, additive, or synergistic effects with respect to antiviral activity. For this reason, we recommend that sponsors evaluate the in vitro antiviral activity of investigational drugs in two- or three-drug combinations with other drugs approved for the same indication. We also recommend completing the in vitro drug combination activity studies of approved drugs with the investigational drug prior to initiation of the clinical trials that will evaluate the efficacy of the combination of the investigational drug with approved drugs. Often subjects are infected with two or more viral diseases (e.g., HIV and HBV or HCV); therefore, we recommend that the in vitro antiviral activity of antiviral drugs used in co-infected patients for different indications be assessed in in vitro combination activity studies.

5. Resistance

a. Selection of resistant virus in vitro

This guidance focuses on resistance to antiretroviral agents caused by mutations in the viral genome that result in reduced phenotypic susceptibility to a given drug product. We recommend that the in vitro selection of resistant viruses to the investigational drug, the phenotypic and genotypic characterization of resistant viruses, and cross-resistance analyses be examined before initiation of clinical studies in patients infected with the particular virus. The completion of in vitro resistance selection studies is recommended to assess the potential of a target virus to mutate and develop reduced susceptibility (i.e., resistance) to the investigational drug. Resistance, as it is used here, is not an absolute term but relative.[3]

Selection in cell culture of virus resistant to the investigational drug can provide insight into whether the genetic threshold for resistance development is high or low. Several factors specific to the investigational drug and the target virus affect the development of resistance (e.g., drug concentration). For some drugs, the development of resistance in the presence of high concentrations of the investigational drug occurs as a result of a single viral mutation whereas others require multiple mutations in the virus. A product with a low genetic threshold may select for resistance with only one or two mutations. In contrast, a product with a high genetic

[3] For an in-depth review of resistance issues relating to HIV-1, see the draft guidance for industry *Role of HIV Drug Resistance Testing in Antiretroviral Drug Development*. When final, this guidance will represent the FDA's current thinking on this topic. For the most recent version of a guidance, check the CDER guidance Web page at http://www.fda.gov/cder/guidance/index.htm.

threshold may require several mutations to result in viral strains with reduced susceptibility. The rate of appearance of mutant viruses is dependent upon the rate of replication of the virus, the number of virus genomes produced, the fidelity of the replicative machinery, and host factors. Consideration of these factors can help in designing tests to detect the appearance in vitro of virus resistant to high concentrations of the drug. For example, many cell culture systems do not produce sufficient virus titers in those instances where multiple mutations are required to develop resistance to high concentrations of the investigational drug. In these instances, serial passage of the virus in cell culture under conditions of increasing concentrations of the investigational drug can lead to the isolation of resistant virus. Sponsors are encouraged to assess the development of resistance in vitro over the concentration range spanning the anticipated in vivo concentration and to determine if the same or different patterns of resistance mutations develop by repeating the selection of variants resistant to the investigational drug several times.

If the targeted virus replicates in a cell culture system, two basic methods can be employed to isolate viruses that have reduced susceptibility to the investigational drug.

- In the first method, a high initial virus inoculum is propagated for several passages at a fixed drug concentration, using multiple cultures to test different concentrations. This approach is useful in identifying drugs for which there is a low genetic barrier to resistance.

- In the second method, virus is passaged in the presence of increasing drug concentrations starting at half the IC_{50} value for the parental virus. Virus production is monitored to detect the selection of resistant virus that is then characterized with respect to genotype and phenotype.

b. Genotypic analysis

Genotypic analysis of resistant viruses selected in vitro determines which mutations might contribute to reduced susceptibility to the investigational drug. Identifying resistance mutations by DNA sequence analysis of the relevant portions of the virus genome can be useful in predicting clinical outcomes and supporting the proposed mechanism of action of the investigational drug. It is highly recommended that sponsors determine the entire coding sequence of the gene for the target protein and compare the pattern of mutations leading to resistance of the investigational drug with the pattern of mutations of other drugs in the same class. In the case of larger viruses, we suggest that the relevant portions of the viral gene targeted by the investigational agent be sequenced and analyzed for mutations that could contribute to drug resistance. It is preferable to characterize resistance pathways in several genetic backgrounds (i.e., strains, subtypes, genotypes) and to obtain isolates during the selection process to identify the order in which multiple mutations appear.

We recommend that sponsors provide the performance characteristics and the details of the methodology of investigational genotypic assays. Sponsors are encouraged to identify sequencing primers, state how many bases can be read from the primer accurately, and define the sensitivity of the genotypic assay used for detecting minority viral subpopulations. It is

important that sponsors define what percentage of the population a mutation has to represent to be detected in their genotypic assay.

c. Phenotypic analysis

Phenotypic analysis determines if mutant viruses have reduced susceptibility to the investigational drug. Once resistance-associated mutations are identified, the ability of each of these mutations to confer phenotypic resistance should be evaluated in a recombinant virus system (e.g., by using site-directed mutagenesis or PCR amplification of relevant portions of virus genome to introduce these mutations into a standard laboratory genetic background). Recombinant viruses should then be tested in vitro for drug susceptibility to determine an IC_{50} value. Phenotypic results can be determined with any standard virus assay: p24 assay, viral RNA assay, RT assay, MTT cytotoxic assay, reporter gene expression, and others. The shift in susceptibility (or fold resistant change) for a viral isolate should be measured by determining the IC_{50} values for the isolate and comparing it to the IC_{50} value of a reference virus done under the same conditions and at the same time. The fold resistant change should be calculated as the IC_{50} value of the isolate/IC_{50} value of the reference strain. We recommend that a well-characterized wild-type laboratory strain grown in cell culture serve as a reference standard.

The utility of a phenotypic assay depends upon its sensitivity (i.e., its ability to measure shifts in susceptibility (fold resistance change) in comparison to reference strains or baseline clinical isolates). Calculating the fold resistant change (IC_{50} value of isolate/IC_{50} value of reference strain) allows for comparisons between phenotypic assays.

Well-characterized genotypic and phenotypic assays should be developed to detect the emergence of resistant virus during the development of candidate drugs. Sponsors can choose to conduct phenotypic and genotypic analyses themselves or send samples to a third party certified by Clinical Laboratory Improvement Amendments (CLIA). Proper handling procedures should be followed for laboratory samples. If the assay is investigational, we recommend that sponsors provide the performance characteristics (e.g., accuracy, precision, limits of detection and quantification, specificity, linearity, range, robustness, stability) of an investigational phenotypic assay and genotypic assay, as well as sources of viruses (e.g., blood, plasma), their storage and stability, and cell culture procedures. For definitions on assay validation, refer to the CDER guidance for industry *Bioanalytical Method Validation* and the ICH guideline *Q2A Text on Validation of Analytical Procedures*. Sponsors are encouraged to use approved assays that are characterized and validated. (If an investigational assay is not performed according to the manufacturer's specifications, the assay results might not be accepted.) We recommend that sponsors be consistent in the genotypic and phenotypic assays used in studies and that the same assay be used for a particular patient throughout the study.

d. Cross-resistance

Antiviral drugs targeting the same protein (typically agents of the same drug class) may develop mutations that lead to reduced susceptibility to one antiviral drug, and can result in decreased or loss of susceptibility to other antiviral drugs in the same drug class. This observation is referred to as cross-resistance. Cross-resistance is not necessarily reciprocal, so it is important to

evaluate both possibilities. For example, if virus X is resistant to drug A and drug B, and virus Y is also resistant to drug A, virus Y may still be sensitive to drug B. Cross-resistance analyses are important in the development of treatment strategies (i.e., establishing the order in which drugs are given). We recommend that the effectiveness of the investigational drug against viruses resistant to other approved drugs in the same class and the effectiveness of approved drugs against viruses resistant to the investigational drug be evaluated by phenotypic analyses. Additionally, we recommend that cross-resistance be analyzed between classes in instances where more than one class of drugs targets a single protein or protein complex (e.g., nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs), which both target the HIV-encoded reverse transcriptase). We recommend that multiple clinical isolates be examined by phenotypic assays with the investigational drug and clinical isolates representative of the breadth of diverse mutations and combinations known (if known) to confer reduced susceptibility. If phenotyping is performed in cell lines, the IC_{50} value obtained in the cell line should be validated with clinical isolates.

IV. PROPOSAL FOR MONITORING RESISTANCE DEVELOPMENT

Given the importance of drug resistance in treatment decisions and the need to disseminate to health care providers information about an antiviral drug's resistance profile, it is strongly recommended that comprehensive resistance testing be undertaken in all phases of drug development. Crucial decisions in protocol design and drug development plans often hinge on resistance and cross-resistance data.

For some viruses, measurements of changes in viral concentrations are accepted endpoints to determine clinical effectiveness of antiviral drugs. Sponsors can choose to quantify viral loads themselves or send samples to companies that have previously been certified by CLIA. In the latter case, proper handling procedures should be followed for laboratory samples. If the antiviral assay has not been previously reviewed and found acceptable by the FDA, we recommend that the sponsor provide a complete description of the methodology and the quantitative assay performance characteristics including upper and lower limits of detection, linearity, and variability and the sources of viruses (e.g., blood, plasma), their storage and stability, and cell culture procedures. Assay results from an assay that is not performed to the manufacturer's specifications might not be accepted. We recommend that sponsors consistently use the same assay for any particular analysis or measurement in studies and that the same assay be used for a particular patient throughout the study.

Prior to the initiation of clinical efficacy studies, we recommend that sponsors submit a plan to monitor the development of resistant viruses in the clinical studies. We recommend including these plans in the nonclinical reports and the overall clinical development plan in the IND. The resistance monitoring plan should include:

- A description of the assays that will be used to monitor viral loads
- How the assays will be performed
- The methods for sample collection and storage
- The methods for sample handling (frozen or ambient)

- The genotypic and phenotypic assays that will be used
- Genotypic and phenotypic assay protocols
- Time points that samples for viral loads, genotypic and phenotypic assays, and other resistance analyses will be collected (i.e., baseline, week 24, week 48, discontinuations)

Phenotypic and genotypic results are used to define treatment options and to predict the utility of treating an individual with an investigational drug. Genotypic analysis of virus isolates from patients failing to respond to therapy or undergoing viral rebound can help identify mutations that contribute to reduced susceptibility to the investigational drug. In addition, genotypic and phenotypic analyses of baseline isolates are encouraged to determine response to therapy outcomes based on baseline mutations/polymorphisms and baseline phenotypic drug susceptibilities. We recommend submitting plans for genotypic and phenotypic baseline studies and resistance substudies early in drug development.

We suggest that genotypic and phenotypic analyses of baseline and post-treatment isolates be completed in a timely manner to characterize the resistance profile of the investigational drug and its cross-resistance potential with other antiviral drugs. Sponsors are strongly encouraged to collect (at a minimum) phenotype and genotype data for baseline isolates from all patients and endpoint isolates from all virologic failures and discontinuations (not suppressed). *Virologic failures*[4] are protocol defined but for HIV-1 studies often include: 1) Rebound: confirmed (two consecutive) plasma viral load values greater than Lower Limit of Quantification (LLOQ) after achieving confirmed level below the LLOQ during the treatment phase; 2) Never Suppressed: viral load levels never achieve confirmed suppression with at least 48 weeks of randomized treatment; or 3) Insufficient Viral Response: viral load levels never achieve confirmed suppression and the investigator identifies insufficient viral load response as the reason for treatment discontinuation prior to week 48. Sponsors with investigational drugs for other viral diseases can solicit advice from the FDA early in drug development on definitions of clinical response/failure and plans to monitor resistance in clinical studies. We recommend that sponsors consult with the DAVDP for detailed descriptions and examples of how to submit clinical viral resistance data. The DAVDP has templates for submitting resistance data for HIV, HBV, and HCV. (See Appendixes 1-3.)

V. VIROLOGY STUDY REPORTS

Complete virology study reports are extensive and should include the raw and analyzed data as well as all the information necessary to evaluate the procedures used to obtain those data. Virology study reports should convey information on nonclinical studies, clinical antiviral activity of the investigational drug, resistance development to the investigational drug in treated patients, and cross-resistance with other drugs in the same drug class. The format of a virology study report should be similar to that of a scientific publication and typically should include the following sections: summary, introduction, materials and methods, results, and discussion. The methods section should describe all the protocols employed and include a description of the

[4] This definition is based on HIV-1 clinical studies. Other definitions can be used and discussed with the DAVDP.

statistical analyses used. Complete virology study reports on nonclinical and clinical studies can be submitted upon their completion and need not be held until submission of the NDA.

VI. SUMMARY

The nonclinical virology section of this guidance identifies studies relevant to development of antiviral drugs for the treatment of viral infections. The goal of this guidance is to stimulate the generation of more complete analyses for antiviral drug products. These analyses provide data that support the introduction of an investigational drug into humans, and provide data necessary for determining dose/response relationships, designing clinical trials, and selecting appropriate patient populations. Thus, these studies have an effect on the therapeutic success of a given product. Because the nonclinical in vitro virology studies provide useful information for the design of in vivo studies and can be predictive of the development of resistant viruses in vivo, we recommend conducting nonclinical studies before the initiation of phase 1 clinical studies and examining the analysis of in vitro selected resistant viruses before initiation of clinical studies in patients infected with the particular virus. The preceding sections of this guidance include recommendations for how and when to perform nonclinical virology studies. Such information could potentially be included in drug labeling to facilitate appropriate prescribing of products and to maximize the chance for therapeutic success.

APPENDIX 1: TEMPLATE FOR SUBMITTING HIV RESISTANCE DATA

One dataset combines patient data, endpoint data, genotypic data, and phenotypic data. There are a number of ways datasets can be subdivided (i.e., by clinical study, baseline isolates, or virologic failure isolates) and it is recommended that this be discussed with the DAVDP before submission.

For each study, we recommend constructing datasets as SAS transport files containing the following information:

- One record (row) per patient per isolate (e.g., baseline, failure, and other time points).
- Data in columns (with suggested column headings shown below) on all isolates.
- Genotypic data for (at a minimum) baseline isolates of all patients and the endpoint isolates of virologic failures and discontinuations — on the corresponding record for each patient isolate.
- Phenotypic data for (at a minimum) baseline isolates and the endpoint isolates of virologic failures and discontinuations — on the corresponding record for each patient isolate.

Virologic failures (other definitions can be used and discussed with the DAVDP)[5] — 1) Rebound: confirmed (two consecutive) plasma HBV DNA values greater than or equal to 1 $\log_{10}$ copies/mL increase in serum HBV DNA from the on treatment nadir; 2) Never Suppressed: plasma HBV DNA levels never achieve confirmed suppression with at least 48 weeks of randomized treatment; or 3) Insufficient Viral Response: plasma HBV DNA levels never achieve confirmed suppression and investigator identifies the reason for treatment discontinuation prior to week 48 due to insufficient viral load response: nonresponders and rebounders.

Information to Include with Suggested Column Headings[6]

I. Patient Data

- Patient identification number
- Isolate (e.g., baseline, week 24, week 48, discontinuation. Multiple isolates numbered (e.g., failure 1, failure 2))
- Date of isolate
- Previous therapeutic agents where available
- Treatment group

[5] The specific criteria for defining virologic failures should be discussed with the DAVDP (Division of Antiviral Drug Products). This definition of virologic failures is intended to be used *only* for the identification of patients from whom isolates should be collected for resistance testing and is *not* intended to be used as a clinical or virologic endpoint in outcome analyses. Isolates from patients with documented clinical progression should also be included.

[6] In the SAS transport files, column headings can be given abbreviated column names to fit the SAS format; however, it is suggested that a description of column names be provided to the reviewer in the submission.

II. Endpoint Data

- HIV RNA (copies/mL) at baseline
- HIV RNA (copies/mL) at predefined time points (e.g., week 24 and week 48)
- HIV RNA (copies/mL) at time of loss of virologic response or discontinuation due to adverse event
- Endpoint assessment (e.g., mean log change in viral load from baseline)
- Other endpoint assessments (e.g., DAVG, mean log change in viral load from baseline)
- Indication of data censored for reasons other than virologic failure (e.g., discontinuation due to adverse event)
- Outcome (i.e., responder, virologic failure, discontinuation while suppressed, discontinuation before achieving viral suppression)
- Reason for discontinuation (i.e., adverse event, pregnancy) or failure (i.e., never suppressed, rebound)
- HIV RNA (copies/ml) from additional time points can be included.

III. Genotypic Data (for baseline isolates of all patients and endpoint isolates from virologic failures and discontinuations)

- Clade
- Genotype for the RT, protease and gp160 (for agents targeting entry only), one amino acid per column with the wild-type (WT) amino acid as column heading. Changes from WT standard sequence indicated (i.e., blanks indicate no change).

Example (This example highlights how genotype information can be displayed but does not include all column headings as previously suggested.)

Patient #	Isolate	V82	N83	I84	I85	G86	R87	N88	L89	L90
001	BL							S		M/L
001	WK48			V				S		M
002	BL	A/T		V				D		M
002	WK48	T		V						M
003	BL	T		V						
004	BL			V						M

BL = baseline
WK48 = week 48

IV. Protease Cleavage Sites (for protease inhibitors only)

- p2/NC protease cleavage site: show amino acid and position of cleavage site of WT in column headings (as above for genotype) and indicate amino acid change if mutant
- NC/p1 Gag cleavage sites: show amino acid and position of cleavage site of WT in column headings (as above for genotype) and indicate amino acid change if mutant

- p1/p6 Gag cleavage sites: show amino acid and position of cleavage site of WT in column headings (as above for genotype) and indicate amino acid change if mutant

V. Phenotypic Data (minimally for baseline isolates and endpoint isolates from virologic failures and discontinuations)

- Candidate drug
  - Baseline IC_{50} value for candidate drug
  - IC_{50} value of reference strain for candidate drug
  - Fold change of baseline IC_{50} value compared to IC_{50} value of reference strain of candidate drug
  - IC_{50} value at time of endpoint assessment or failure for candidate drug
  - Fold change values in IC_{50} value at time of endpoint assessment or failure compared to reference strain for candidate drug
  - Fold change in IC_{50} value at time of endpoint assessment or failure compared to baseline for candidate drug

- Approved/investigational anti-HIV agents (List first agents in the same class in alphabetical order followed by agents with the same target protein in alphabetical order. End with agents outside drug class in alphabetical order.)
  - Fold change in IC_{50} value of baseline compared to reference strain for all approved/investigational anti-HIV agents
  - Fold change in IC_{50} value at time of endpoint assessment or failure compared to reference strain for each of the approved/investigational anti-HIV agents
  - Fold change in the IC_{50} value at time of endpoint assessment or failure compared to baseline for each of the approved/investigational anti-HIV agents

Example (This example highlights how phenotype information can be displayed but does not include all column headings as previously suggested.)

Sample	Drug X				Other Drugs in Same Drug Class*		Other Drugs Outside Drug Class*	
	IC_{50} value Drug X	Ref strain IC_{50} value Drug X	Δ resis from ref Drug X	Δ resis from BL Drug X	Δ resis from ref Drug Y	Δ resis from BL Drug Y	Δ resis from ref Drug A	Δ resis from BL Drug A
Baseline								
Endpoint								

Drug X = candidate drug

Δ resis = fold resistance change, e.g.: $\frac{IC_{50} \text{ value of baseline sample with Drug X}}{IC_{50} \text{ value of reference strain with Drug X}}$

Ref strain = reference strain (or WT)

Endpoint = predefined time point for endpoint assessment (e.g., week 24, week 48, failure or discontinuation)

*Note: Include the Δ resis from ref and Δ resis from BL for all approved anti-HIV drugs

VI. Column with Total Number of PI Mutations in Patient Isolate (for baseline and endpoint isolates)

PI mutations to be counted include changes at amino acid D30, V32, M361, M46, G48, I50, I54, G73, V82, I84, N88, L90, and any additional amino acid changes in PR that are important/relevant to the study drug as shown in vitro or in clinical trials. Discuss with the DAVDP in advance for agreement on the total number of mutations.

VII. Column with Total Number of NRTI Mutations in Patient Isolate (for baseline and endpoint isolates)

RT mutations to be counted include changes at amino acid M41, E44, K65, D67, T69, K70, L74, Y115, V118, M184, L210, T215, and any additional amino acid changes in RT that are important/relevant to the study drug as shown in vitro or in clinical trials. Discuss with the DAVDP in advance for agreement on the total number of mutations.

VIII. Column with Total Number of NNRTI Mutations in Patient Isolate (for baseline and endpoint isolates)

RT mutations to be counted include changes at amino acid A98, L100, K103, V106, V108, Y181, Y188, G190, P225, M230, P236 and any additional amino acid changes in RT that are important/relevant to the study drug as shown in vitro or in clinical trials. Discuss with the DAVDP in advance for agreement on the total number of mutations.

IX. Co-Receptor Usage (for all agents targeting entry)

- Co-receptor usage of baseline isolates. Indicate R5, X4, D for dual-tropic, M for mixed-tropic, or D/M if the assay cannot distinguish between dual or mixed, in a column.
- Baseline R5 assay value
- Baseline X4 assay value
- Co-receptor usage of virologic failures and end-of-study isolates (on therapy). Indicate R5, X4, D for dual-tropic, M for mixed-tropic, or D/M if the assay cannot distinguish between dual or mixed, in a column.
- R5 assay value at failure/end of study
- X4 assay value at failure/end of study

APPENDIX 2: TEMPLATE FOR SUBMITTING HBV RESISTANCE DATA

One dataset combines patient data, endpoint data, genotypic data, and phenotypic data. There are a number of ways datasets can be subdivided (i.e., by clinical study, baseline isolates, or virologic failure isolates) and it is recommended that this be discussed with the DAVDP before submission.

For each study, we recommend constructing datasets as SAS transport files containing the following information:

- One record (row) per patient per isolate (e.g., baseline, failure, and other time points).
- Data in columns (with suggested column headings shown below) on all isolates.
- Genotypic data for (at a minimum) baseline isolates of all patients and the endpoint isolates of virologic failures and discontinuations — on the corresponding record for each patient isolate.
- Phenotypic data for (at a minimum) baseline isolates and the endpoint isolates of virologic failures and discontinuations — on the corresponding record for each patient isolate. We recognize the difficulty of phenotypic analysis of HBV. Sponsors are strongly encouraged to collect and store appropriately samples for later analysis if warranted. If the pathway to resistance as defined by genotypic analysis is straightforward (e.g., lamivudine), phenotypic analysis may not be necessary. We recommend that sponsors consult with the DAVDP to determine whether phenotypic analysis should be conducted. Samples should be collected from rebound and failure isolates for phenotypic analysis when the subject is on study drug.

Virologic failures (other definitions can be used and discussed with the DAVDP)[7] — 1) Rebound: confirmed (two consecutive) plasma HBV DNA values greater than or equal to 1 $\log_{10}$ copies/mL increase in serum HBV DNA from the on treatment nadir; 2) Never Suppressed: plasma HBV DNA levels never achieve confirmed suppression with at least 48 weeks of randomized treatment; or 3) Insufficient Viral Response: plasma HBV DNA levels never achieve confirmed suppression, and investigator identifies the reason for treatment discontinuation prior to week 48 due to insufficient viral load response: nonresponders and rebounders.

Information to Include with Suggested Column Headings[8]

I. Patient Data

- Patient identification number

[7] The specific criteria for defining virologic failures should be discussed with the DAVDP (Division of Antiviral Drug Products). This definition of virologic failures is intended to be used *only* for the identification of patients from whom isolates should be collected for resistance testing and is *not* intended to be used as a clinical or virologic endpoint in outcome analyses. Isolates from patients with documented clinical progression should also be included.

[8] In the SAS transport files, column headings can be given abbreviated column names to fit the SAS format; however, it is suggested that a description of column names be provided to the reviewer in the submission.

- Isolate (e.g., baseline, week 24, week 48, discontinuation. Multiple isolates numbered (e.g., failure 1, failure 2))
- Date of isolate
- Days of study drug treatment on day isolate obtained
- Days off study drug treatment on day isolate obtained
- Previous therapeutic agents
- Treatment group

II. Endpoint Data

- HBV DNA (log_{10} copies/mL) at baseline
- HBV DNA (log_{10} copies/mL) at predefined time points (e.g., week 24 and week 48)
- HBV DNA (log_{10} copies/mL) at time of loss of virologic response or discontinuation due to adverse event
- Endpoint assessment (e.g., log_{10} change in viral load from baseline)
- Indication of data censored for reasons other than virologic failure (e.g., discontinuation due to adverse event)
- HBV DNA (log_{10} copies/ml) from additional time points can be included.

Note: We recommend that sponsors analyze HBV DNA with a sensitive and specific HBV DNA assay with a lower limit of quantification less than 1,000 copies/mL.

III. Genotypic Data (for baseline isolates of all patients and endpoint isolates from virologic failures and discontinuations)

- HBV subtype
- Genotype information for all the RT or relevant coding region sequenced, one amino acid per column with the wild-type (WT) amino acid as column heading identified using the one amino acid abbreviation. Changes from the WT standard sequence should be indicated (i.e., blanks indicate no change) and known polymorphic amino acid residues flagged in the column heading.

Example (This example highlights how genotype information can be displayed but does not include all column headings as previously suggested.)

Patient #	Isolate	Subtype	rtY203	rtM204	rtD205	rtD206
001	BL	A				
001	WK48	A		V		
002	BL	C				
002	WK48	C		I		
003	BL	A				
004	BL	B		V		

BL = baseline
WK48 = week 48

IV. Phenotypic Data (minimally for baseline isolates and endpoint isolates from virologic failures and discontinuations)

- Candidate drug
  - Baseline IC_{50} value for candidate drug
  - IC_{50} value of reference strain for candidate drug (a widely available standard laboratory strain is recommended as the reference strain)
  - Fold change of baseline IC_{50} value compared to IC_{50} value of reference strain of candidate drug
  - IC_{50} value at time of endpoint assessment or failure for candidate drug
  - Fold change values in IC_{50} value at time of endpoint assessment or failure compared to reference strain for candidate drug
  - Fold change in IC_{50} value at time of endpoint assessment or failure compared to baseline for candidate drug

- Approved/investigational agents in the same class (cross-resistance, to be determined in cases of demonstrated reduced susceptibility to the treatment drug)
  - Fold change in IC_{50} value of baseline compared to reference strain for each of the approved/investigational agents in the same class
  - Fold change in IC_{50} value at time of endpoint assessment or failure compared to reference strain for each of the approved/investigational agents in the same class
  - Fold change in the IC_{50} value at time of endpoint assessment or failure compared to baseline for each of the approved/investigational agents in the same class

Example (The example highlights how phenotype information can be displayed.)

Sample	Drug X				Other Drugs in Same Drug Class*	
	IC_{50} value Drug X	Ref strain IC_{50} value Drug X	Δ resis from ref Drug X	Δ resis from BL Drug X	Δ resis from ref Drug Y	Δ resis from BL Drug Y
Baseline						
Endpoint						

Drug X = candidate drug
BL = baseline
Endpoint = predefined time point for endpoint assessment (e.g., week 24, week 48, failure or discontinuation)
Δ resis = fold resistance change, e.g.: $\frac{IC_{50} \text{ value of baseline sample with Drug X}}{IC_{50} \text{ value of reference strain with Drug X}}$
Ref strain = reference strain (or WT)
*Note: Include the Δ resis from ref and Δ resis from BL for all approved anti-HBV drugs

APPENDIX 3: TEMPLATE FOR SUBMITTING HCV RESISTANCE DATA

One dataset combines patient data, endpoint data, genotypic data, and phenotypic data. There are a number of ways datasets can be subdivided (i.e., by clinical study, baseline isolates or virologic failure isolates) and it is recommended that this be discussed with the DAVDP before submission.

For each study, we recommend constructing datasets as SAS transport files containing the following information:

- One record (row) per patient per isolate (e.g., baseline, failure, and other time points).
- Data in columns (with suggested column headings shown below) on all isolates.
- Genotypic data for (at a minimum) baseline isolates of all patients, the endpoint isolates of virologic failures and discontinuations, and follow-up for sustained virologic response — on the corresponding record for each patient isolate.
- Phenotypic data for (at a minimum) baseline isolates, the endpoint isolates of virologic failures and discontinuations, and follow-up for sustained virologic response — on the corresponding record for each patient isolate. We recognize the difficulty of phenotypic analysis of HCV. Sponsors are strongly encouraged to collect and store appropriately samples for later analysis if warranted. If the pathway to resistance as defined by genotypic analysis is straightforward, phenotypic analysis may not be necessary. We recommend that sponsors consult with the DAVDP to determine whether phenotypic analysis should be conducted. Samples should be collected from rebound and failure isolates for phenotypic analysis when the subject is on study drug.

Virologic failures (other definitions can be used and discussed with the DAVDP)[9] — 1) Rebound: confirmed (two consecutive) plasma HBV DNA values greater than or equal to 1 $\log_{10}$ copies/mL increase in serum HBV DNA from the on treatment nadir; 2) Never Suppressed: plasma HBV DNA levels never achieve confirmed suppression with at least 48 weeks of randomized treatment; or 3) Insufficient Viral Response: plasma HBV DNA levels never achieve confirmed suppression and investigator identifies the reason for treatment discontinuation prior to week 48 due to insufficient viral load response: nonresponders and rebounders.

Information to Include with Suggested Column Headings[10]

I. Patient Data

[9] The specific criteria for defining virologic failures should be discussed with the DAVDP (Division of Antiviral Drug Products). This definition of virologic failures is intended to be used *only* for the identification of patients from whom isolates should be collected for resistance testing and is *not* intended to be used as a clinical or virologic endpoint in outcome analyses. Isolates from patients with documented clinical progression should also be included.

[10] In the SAS transport files, column headings can be given abbreviated column names to fit the SAS format; however, it is suggested that a description of column names be provided to the reviewer in the submission.

- Patient identification number
- Isolate (e.g., baseline, week 24, week 48, discontinuation. Multiple isolates numbered (e.g., failure 1, failure 2))
- Date of isolate
- Days of study drug treatment on day isolate obtained
- Days off study drug treatment on day isolate obtained
- Previous therapeutic agents where available
- Treatment group

II. Endpoint Data

- HCV RNA (log_{10} copies/mL) at baseline
- HCV RNA (log_{10} copies/mL) at predefined time points (e.g., week 24 and week 48)
- HCV RNA (log_{10} copies/mL) at time of loss of virologic response or discontinuation due to adverse event
- Endpoint assessment (e.g., log_{10} change in viral load from baseline)
- Follow-up for determination of sustained virologic response
- Indication of data censored for reasons other than virologic failure (e.g., discontinuation due to adverse event)
- HCV RNA (log_{10} copies/ml) from additional time points can be included.

Note: We recommend that sponsors analyze HCV RNA with a sensitive and specific HCV RNA assay with lower limits of quantification in the range of less than 100 copies/mL.

III. Genotypic Data (for baseline isolates of all patients and endpoint isolates from virologic failures and discontinuations)

- HCV subtype (e.g., 1a, 1b, 2)
- Genotype information for all the replicases or relevant coding region sequenced, one amino acid per column with the wild-type (WT) amino acid as column heading identified using the one amino acid abbreviation. Changes from WT standard sequence indicated (i.e., blanks indicate no change) and known polymorphisms are indicated with an asterisk.

Example (This example highlights how genotype information can be displayed but does not include all column headings as previously suggested.)

Patient #	Isolate	Subtype	E414	F415	D416	L417
001	BL	1				
001	WK48	1		Y		
001	FU	1				
002	BL	1				
002	WK48	1		Y		
002	FU	1		Y		

BL = baseline
WK48 = week 48
FU = follow-up

IV. Phenotypic Data (minimally for baseline isolates, endpoint, and follow-up isolates from virologic failures and discontinuations)

- Candidate drug
  - Baseline IC_{50} value for candidate drug
  - IC_{50} value of reference strain for candidate drug (a widely available standard lab strain is recommended as the reference strain)
  - Fold change of baseline IC_{50} value compared to IC_{50} value of reference strain of candidate drug
  - IC_{50} value at time of endpoint assessment or failure for candidate drug
  - Fold change values in IC_{50} value at time of endpoint assessment or failure compared to reference strain for candidate drug
  - Fold change in IC_{50} value at time of endpoint assessment or failure compared to baseline for candidate drug
  - IC_{50} value at follow-up for candidate drug
  - Fold change values in IC_{50} value at follow-up compared to reference strain for candidate drug
  - Fold change in IC_{50} value at follow-up compared to baseline for candidate drug

- Approved/investigational agents in the same class
  - Fold change in IC_{50} value of baseline compared to reference strain for each of the approved/investigational agents in the same class
  - Fold change in IC_{50} value at time of endpoint assessment or failure compared to reference strain for each of the approved/investigational agents in the same class
  - Fold change in the IC_{50} value at time of endpoint assessment or failure compared to baseline for each of the approved/investigational agents in the same class

Example (The example highlights how phenotype information can be displayed.)

	Drug X				Other Drugs in Same Drug Class*	
Sample	IC_{50} value Drug X	Ref strain IC_{50} value Drug X	Δ resis from ref Drug X	Δ resis from BL Drug X	Δ resis from ref Drug Y	Δ resis from BL Drug Y
Baseline						
Endpoint						

Drug X = candidate drug
BL = baseline
Endpoint = predefined time point for endpoint assessment (e.g. week 24, week 48, failure or discontinuation)
Δ resis = fold resistance change, e.g.: $\frac{IC_{50} \text{ value of baseline sample with Drug X}}{IC_{50} \text{ value of reference strain with Drug X}}$
Ref strain = reference strain (or WT)
*Note: Include the Δ resis from ref and Δ resis from BL for all approved anti-HCV drugs